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Work described in past progress reports led to the conclusion that the post-synaptic glutamate receptors which mediate fast, excitatory transmission in mammalian brain are the sites at which the changes responsible for LTP occur. Moreover, pharmacological and physiological experiments indicated that the nature of the change involved a modification of receptor channel kinetics. Modelling studies, incorporating this information into a biologically realistic simulation of the receptor, resulted in a specific hypothesis about which the channel opens and closes (see Progress Report, 1992-1993). During the past year, experimental work was carried out to test this hypothesis. This involved hippocampal slices in which fast, excitatory responses were isolated by pharmacologically blocking inhibitory conductances and post-synaptic spiking. The synaptic responses in these "disinhibited" slices are simple reflections, modified by dendritic filtering, of AMPA receptor mediated currents.

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The goals of this research program are to *i)* identify the cellular changes responsible for long-term potentiation (LTP) and *ii)* apply this information to the development of memory enhancing drugs. Results in each area obtained during the past year are described below.

Role of AMPA-type glutamate receptors in the expression of LTP

Work described in past Progress Reports led to the conclusion that the post-synaptic glutamate receptors which mediate fast, excitatory transmission in mammalian brain are the sites at which the changes responsible for LTP occur. Moreover, pharmacological and physiological experiments indicated that the nature of the change involved a modification of receptor channel kinetics. Modelling studies, incorporating this information into a biologically realistic simulation of the receptor, resulted in a specific hypothesis about which kinetic parameters are altered so as to produce potentiation; i.e., the rate at which the channel opens and closes (see Progress Report, 1992-1993). During the past year, experimental work was carried out to test this hypothesis. This involved hippocampal slices in which fast, excitatory responses were isolated by pharmacologically blocking inhibitory conductances and post-synaptic spiking. The synaptic responses in these "disinhibited" slices are simple reflections, modified by dendritic filtering, of AMPA receptor mediated currents. The experiment involved a comparison of how three manipulations affected the waveform of the isolated response: *i)* a drug (aniracetam) known to prolong the mean open time of the receptors, *ii)* LTP and *iii)* LTP and aniracetam combined. In agreement with our previous reports, LTP was found to produce a characteristic distortion of the waveform of the synaptic response. Aniracetam's effects on the waveform were then shown to be substantially altered by the prior induction of LTP, as predicted by the hypothesis that potentiation effect had changed the target system of the drug (i.e., channel kinetics). Moreover, the form of the interaction between aniracetam and LTP was readily accounted for by the computer simulation of the AMPA receptor when LTP was modelled as an increase in the rate constants for channel opening and closing (Kolta *et al.*, 1994).

The mounting evidence that LTP is due to a specific change in AMPA receptor properties has led to a gradual switch in the laboratory's emphasis from physiological analyses of the potentiation effect to a biochemical search for the synaptic factors responsible for the receptor modification. Several studies of this kind were completed in the past year. First, it was established that solubilization of AMPA receptors from synaptic membrane fractions profoundly increases their affinity for agonists but not antagonists (Hall *et al.*, 1993a) and that this effect is not due to a shift of the receptor into a desensitized state (Hall *et al.*, 1993b). It thus appears that some element in the synaptic environment maintains the AMPA receptor in a particular state; it should be noted that this extreme dependency on the synaptic region is not reported for other ionotropic receptors (e.g., the

NMDA subclass of glutamate receptors co-localized with AMPA receptor; GABA receptors). Second, it was shown that treatments which disrupt protein-protein interactions (UV radiation, thiol reducing agents) convert membrane bound AMPA receptors into the high affinity state characteristic of solubilized receptors (Hall *et al.*, 1994a). This result strongly suggests that a protein present in high concentrations in the synaptic zone regulates the properties of the AMPA receptor. Third, heparin converts soluble receptors into the low affinity state characteristic of receptors bound to synaptic membranes (Hall *et al.*, 1994b). Heparin is known to cause protein aggregation and evidence was obtained from size exclusion columns and ultracentrifugation that it has this effect on AMPA receptors. Immunoprecipitation experiments showed that no proteins were tightly associated with AMPA receptors after heparin treatment. Accordingly, we now advance the hypothesis that clustering (aggregation) of AMPA receptors changes their functional properties and that this is the mechanism whereby the synaptic environment controls receptor operation.

Previous work from this laboratory had indicated that cytoskeletal/structural changes occur in the synaptic region in association with LTP. It can be assumed that such modifications affect the distribution of transmembrane proteins such as the AMPA receptor; if so, then the structural changes would also alter the probability (frequency) of receptor-receptor interactions and hence alter the properties of the receptors.

Evidence has been obtained that the induction of stable LTP involves the activation of calcium sensitive protease calpain (see previous Progress Reports). New technologies involving translational suppression of calpain in cultured slices and antibodies specific to a calpain-mediated protein breakdown product have added further support to hypothesis. These experiments should be completed in the next several months and the results described in the next Progress Report.

Facilitation of AMPA receptors enhances memory

As described in last year's Progress Report, AFOSR funds were used to design, synthesize, and test a new class of drugs that act on the kinetics of the AMPA receptor channel so as to enhance the ion currents it passes. It was known from earlier work in the laboratory that drugs that produce such effects substantially reduce the amount of afferent activity needed to induce robust LTP in brain slices (see Progress Report, 1992-1993); given the evidence linking LTP to memory, it was reasonable to assume that drugs producing such effects in behaving animals would potently facilitate the encoding of memory. This prediction was confirmed in the past year using three members of a family of compounds that have been developed. (The name "Ampakines" has been used to describe this family; when more is known about structure-activity relationships, it should be possible to substitute a more descriptive biochemical term.) Before summarizing the behavioral data, it is appropriate to list results obtained during the past year regarding the physiological effects of the drugs.

Excised patch studies have shown that Ampakines prolong the duration of AMPA receptor-mediated currents in a fashion that has a very rapid onset and washout (Arai *et al.*, 1994). Comparable effects are observed on synaptic responses recorded from *in vitro* brain

slices (Staubli *et al.*, 1994a). PET scan experiments using "C-labelled" drug indicated that the drugs reach the rat brain within one minute of the intraperitoneal injection and equilibrate at high concentrations within 2-5 minutes; physiological recording from freely moving rats confirmed that the drugs have a very rapid onset of action after peripheral administration and remain effective for 90-120 minutes. These latter studies also revealed that Ampakines produce the expected facilitation and prolongation of synaptic responses *in situ*; these drugs are thus the first compounds known to enhance fast, excitatory transmission in brain. Finally, chronic recording experiments have demonstrated that the AMPA drugs markedly reduce the number of afferent stimulation bursts needed to induce LTP in behaving animals (Staubli *et al.*, 1994b).

The drugs have been shown in several studies to improve memory across a variety of behavioral tasks including the following:

i) recent memory in radial mazes (Granger *et al.*, 1993; Staubli *et al.*, 1994a,b),

ii) olfactory match to sample (Staubli *et al.*, 1994b),

iii) eyeblink conditioning to a tone (Shors *et al.*, 1994),

iv) two odor discriminations (Staubli *et al.*, 1994a; Larson *et al.*, in prep.),

v) conditioned fear responses (LeDoux *et al.*, 1994).

Projects i, ii, and iv were supported by AFOSR, iii and v are studies done at other institutions. In certain of the above experiments, it was possible to measure the effects of the drugs on performance during acquisition (Granger *et al.*, 1993; Larson *et al.*, *in prep*; Shors *et al.*, 1994). These analyses have shown that the AMPA drugs at dosages which do not affect indices to arousal, or performance times during acquisition, cause a marked enhancement of retention. The compounds are now being tested in laboratories around the country and there should shortly be very large amount of information available about the varieties of memory they facilitate. Finally, the AMPA drugs have been tested for chronic use and found to be safe and efficacious over periods of weeks (unpublished).

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